

Hypoglycemic effect of leafy vegetables from Ankole and Teso sub-regions of Uganda: preclinical evaluation using a high fat diet-streptozotocin model

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Abstract

Background: The hypoglycemic effect of the most frequently eaten leafy vegetables in Ankole and Teso sub-regions of Uganda, *Hibiscus sabdariffa* L. (Hs), *Vigna unguiculata* L. Walp (Vu) and *Solanum nigrum* L. (Sn) was studied in an attempt to explain the remarkable difference in prevalence of hyperglycemia observed in the two sub-regions.

Methods: Phytochemical screening was done and the phytochemicals in the Aqueous leaf extracts (ALEs) of Hs, Vu and Sn were quantified by means of UV-visible spectrophotometer using the standard calibration graph. The phytochemical concentration was given as standard equivalent of the vegetable fresh weight. The extracts were then tested for their hypoglycemic activity in high fat diet-streptozotocin (HFD-STZ) induced diabetic Wistar rats. These extracts were administered to diabetic rats at 100- and 200 mg/kg/body weight, orally once a day for 12 days. Glibenclamide (a standard drug used to treat diabetes mellitus) was similarly given to a positive control group to support the results at a dose of 5 mg/kg/body weight. Diabetic and normal control groups received vehicle (distilled water) daily for the same period of time.

Results: More phytochemical compounds were found in aqueous than methanol solvent. The highest quantity of total phenolic (TP) compounds was found in Hs 66.11 ± 0.19 Gallic Acid Equivalent (GAE) ($\mu\text{g}/\text{mg}$ fresh weight), highest quantity of total flavonoids (TF), total tannins (TT) and total alkaloids (TA) were found in Sn (71.35 ± 0.14 Quercetin equivalent of flavonoids (QEF), 172.17 ± 8.69 GAE and 119.71 ± 3.64 Atropine equivalent ($\mu\text{g}/\text{mg}$, fresh weight) respectively, whereas the total saponins (TS) were highest in Vu 125.78 ± 0.47 Diosgenin equivalent of Saponin per mg of fresh weight of the vegetable.

Conclusions: The ALEs of Hs lowered the fasting blood glucose (FBG) levels most significantly (65%) at the dose of 200 mg/kg; Sn lowered the FBG by 57%, while Vu lowered the FBG by 43%, compared to the diabetic control. Vegetable hypoglycemic effects were comparable with glibenclamide (66%). Animal body weights showed insignificant increases by the end of the study. These vegetables can be considered effective in prevention and management of hyperglycemia.

Introduction

Hyperglycemia is defined as increased blood glucose levels (above optimal), which is a characteristic feature of a metabolic syndrome called type 2 Diabetes Mellitus (T2DM) [1]. Type 2 diabetes mellitus accounts for the vast majority of diabetes around the world [2]. When hyperglycemia is not well managed, it has dire consequences on health through the development of micro- and macro-vascular complications. These complications have serious effects on the morbidity of diabetics and can lead to mortality [1, 3]. According to the International Diabetes Federation (IDF) the global prevalence of diabetes was 9.3% in 2019 and it was projected to rise to 10.2% by 2030 [4]. The WHO in 2016 reported the prevalence of diabetes mellitus at 8.5% in the adult population, and so over the past few years, it has risen even faster in low- and middle-income countries [2]. The IDF in Uganda recorded a prevalence of

3.6% in 2021 [5] and in 2016, a survey by Bahendeka et al. reported 1.4% [6]. According to 2016 Uganda's country profile, the national mortality proportion by diabetes was 1%, where 2,570 deaths were recorded directly from hyperglycemia [7]. This prevalence is relatively low, and this presents an opportunity to intervene before it increases. Type 2 DM is caused by an array of metabolic disorders arising from an interplay of genetic and environmental factors. These factors if not attended to, can lead to a physiological impairment of insulin secretion by the pancreatic β -cells and its action on the target tissues [8]. This latter scenario manifests through impaired glucose tolerance, hyperglycemia, which if it persists progresses to diabetes [8, 9]. This progression is not inevitable, and can be prevented by lifestyle modification and medication [10]. Current antidiabetic therapies are efficacious in normalizing high blood glucose levels in diabetics; however, they possess limitations and challenges such as adverse effects, limited access, and high cost, which have led to non-adherence and poor glycemic control in diabetics [11, 12]. There is thus need for alternative and complementary interventions, of which vegetables present some hypoglycemic evidence due to the nutrient and non-nutrient components they possess [13, 14]. In addition, vegetables seem to be a contextualized alternative for the world's low- and middle-income countries since they are not only an important component in their traditional diets but also are accessible and cheaper [15, 16].

In Uganda, we carried out a survey to document the prevalence of hyperglycemia and the frequently eaten leafy vegetables, and we found Hs, Vu and Sn to be among the frequently eaten traditional leafy vegetables in Ankole and Teso sub-regions [17]. It is noteworthy that there is relatively scanty documentation of animal studies on the leafy parts of these vegetables compared to their other parts such as calyces for Hs, seeds for Vu and berries for Sn. However, the few previous studies in animal model have reported the beneficial effects of Hs, Vu and Sn extracts on blood glucose level and other diabetic parameters, and many researchers suggested that this hypoglycemic effect is due to the phytochemical classes such as polyphenols, flavonoids richly quantified in them [18–20].

Methanol extract of Hs leaves at a dose of 400mg/kg attained 80.5% reduction in blood glucose in Alloxan induced rats [21]. Researchers have popularized the use of plant extract mediated nanoparticles due to their higher solubility in water, biocompatibility, and are devoid of toxic stabilizers. Hs leaf Zinc oxide nanoparticles were able to reduce blood glucose levels in mice up to 59.6% [22]. A methanol extract of a combination of Hs leaves and other medicinal plants (*Piper nigrum* and *Zingiber officinale*) showed a significant reduction of blood glucose compared to Metformin in a study conducted by Ime et al., (2022) [23]. An in-vitro study of the leaf extract of different cultivars of Vu revealed an improvement in diabetic parameters through inhibition of digestive enzymes like α -glucosidase and α -amylase, up to 94% compared to the commercial inhibitor Acarbose. In addition, there was an upregulation of genes expressing glucose transporter 4 [19]. The pathophysiology of T2DM states that increase in intercellular lipids can lead to insulin resistance and consequently hyperglycemia [24], however flavonoids compounds in Vu leaves showed an antilipidemic activity in rabbits [25], suggesting that consumption of Vu leaves in the diet can be a preventive measure for hyperglycemia. Numerous experiments with different solvent leaf extracts of Sn in different animal models have reported a reduction in blood glucose level in either normal or diabetic animals. For example, Sn leaves have been reported to reduce blood

glucose level in Alloxan induced diabetic guinea pigs [26]. The glucose tolerance test with Sn leaf extract mediated Silver nano-particles showed that Sn leaves have an efficacy close to that of glybenclamide in diabetic rats [27]. Likewise Maharam et al., (2010) reported a significant reduction in blood glucose level in normal rats by the aqueous leaf extract of Sn [28].

It is therefore against this background studies that we designed our study to evaluate the three most frequently eaten vegetables: *Hibiscus sabdariffa* L. (Roselle), *Vigna unguiculata* L. Walp (cow peas) and *Solanum nigrum* L. (black nightshade), in Ankole and Teso sub-regions in Uganda for their hypoglycemic effect, to explain the reported marked variance of prevalence in hyperglycemia in these sub-regions according to Bahendeka et al. (2014) population based survey on prevalence and correlates of T2DM in Uganda.

Materials and Methods

Collection of vegetable material and preparation of extracts

Hibiscus sabdariffa L. (Hs) and *Vigna unguiculata* L. Walp (Vu) leaves were collected from Katakwi and Ngora districts (respectively) in Teso sub-region. *Solanum nigrum* L. (Sn) leaves were collected from Mbarara district in Ankole sub-region. The plants were identified by a taxonomist and given specimen identification numbers (*Hibiscus sabdariffa*-51177, *Vigna unguiculata* – 51172 and *Solanum nigrum* – 51174). These vegetable samples were deposited at the National Herbarium at Makerere University, Kampala. The leaves of Hs, Vu and Sn were air-dried under room temperature and the dry leaves were ground into powder using an electric grinder. Powdered leaves (200 g) of each vegetable were extracted by boiling in 2000 ml of distilled water for 15 minutes. The same amount was macerated in 75% methanol for 72 hours with occasional shaking [29]. The vegetable filtrates (extracts) were frozen at -80°C, and then dried in a freeze drier. The dried extracts were stored in a refrigerator at -6°C till use.

Chemicals and instruments

Phytochemical standards (Quercetin, Gallic acid, Diosgenin and Atropine), Folin-Ciocalteu and Streptozotocin (STZ) (CAS-No: 18883-66-4) were purchased from Sigma-Aldrich, German. More chemicals were; Aluminium Chloride, Sodium acetate, Vanillin, Sulphuric acid, Hydrochloric acid, Bromocresol Green. Ultra–Violet/Visible (UV-Vis) 6705 Spectrophotometer (JENWAY). Pure methanol and distilled water from a Milli-Q purification system (Millipore, India). All the chemicals used were of analytical grade.

Phytochemical screening and quantification

Both aqueous and methanol leaf extracts of Hs, Vu and Sn vegetable leaves were screened for phytochemicals [30, 31]. The following reagents and chemicals were used: Alkaloids with Dragendroff's reagent, glycosides with modified Borntrager's test, flavonoids with lead acetate test, phenolic compounds with ferric chloride test, tannins with Braymer's test, phytosterols with Salkowski's test,

coumarins with NaOH test, terpenoids with chloroform and concentrated sulphuric acid, and saponins with the Foam test.

Determination of total flavonoid (TF) content

Total flavonoid content in the VALEs was determined using a modified aluminium chloride colorimetric method [32]. To 1 mL of 1 mg/mL concentration of each VALEs, 3 mL of methanol was added and agitated followed by the addition of 0.2 mL of 10% AlCl_3 solution and 0.2 mL of 1M sodium acetate. Thereafter, the solutions were incubated for 30 min at room temperature in the dark. The absorbance of the resultant solutions was then read at 420 nm using a UV-VIS spectrophotometer. Working solutions of 10–200 $\mu\text{g/mL}$ of Quercetin standard were prepared from the standard concentration of 1 mg/mL and were used to prepare a calibration curve. The concentration of TF compound in each VALEs was determined using an equation $y = 0.0145x - 0.2463$ and $R^2 = 0.9453$ obtained from a standard quercetin graph. The TF content was expressed as microgram (μg) Quercetin Equivalent of Flavonoids (QEF) per milligram (mg) of each vegetable dry weight.

Determination total saponin (TS) content

TS content in VALEs was determined using Vanillin–sulphuric acid assay [33]. To 1 mL of 1 mg/mL concentration of each VALEs was added 0.5 mL of 8% (w/v) vanillin solution, followed by addition of 5 mL of 72% (v/v) Sulphuric acid and thoroughly mixed. The resultant mixtures were then incubated at 60 °C in a shaking water bath for 15 minutes and then cooled in ice-cold water for 5 minutes. The absorbance of the resultant solutions was then read at 550 nm using a UV-vis spectrophotometer. Working solutions of 1–100 $\mu\text{g/mL}$ of Diosgenin standard were used to prepare a calibration curve. The concentration of TS compound in the VALEs was determined using an equation $y = 0.0049x + 0.0505$ and $R^2 = 0.9917$ obtained from a standard Diosgenin graph. The TS content was expressed as μg Diosgenin equivalent of Saponin per mg of vegetable dry material.

Determination total phenolic (TP) content

TP content of VALEs was determined using a modified Folin-Ciocalteu method as described by [20]. To 1 mL concentration of 1 mg/mL VALEs was added 2 mL of 10% (v/v) Folin-Ciocalteu reagent (Sigma-Aldrich, Germany), followed by the addition of 2 mL of 7.5% (w/v) sodium carbonate solution and incubated at 40°C for 30 minutes. The absorbance of the resultant solution was then read at 760 nm. Working solutions of 1-100 $\mu\text{g/mL}$ of Gallic acid were used to prepare a calibration curve for the standard. The concentration of TP compound in the VALEs was determined using an equation $y = 0.0304x - 0.2872$ and $R^2 = 0.9546$ obtained from a standard gallic acid graph. The TP content was expressed as μg Gallic Acid Equivalent (GAE) /mg dry weight.

Determination total tannins (TT) content

TT content was determined using a modified vanillin-HCL assay method [34]. To 1000 μL concentration of 1 mg/mL of VALEs were added 1500 μL of vanillin/methanol (4%) solution and 750 μL of concentrated HCl and allowed to react at room temperature for 1 h. The absorbance at 300 nm was

measured against a blank. Working solutions of 1-100 µg/mL of gallic acid were used to prepare a calibration curve for the standard. The concentration of TT compound in VALEs was determined using an equation $y = 0.0104x + 0.0667$ and $R^2 = 0.848$ obtained from a standard gallic acid graph. The total concentration of condensed tannins was expressed in µg of GAE per milligram dry vegetable sample.

Determination total alkaloids (TA) content

VALEs were dissolved in 2N HCL acid and then filtered. One 1 mL of 1 mg/ml concentration of each vegetable solution was transferred to separating funnel and washed with 10 mL chloroform (3 times). The pH of this solution was adjusted to neutral with 0.1 N NaOH. Thereafter, 5 mL of Bromocresol Green solution and 5 ml of phosphate buffer were added to these solutions. The mixtures were shaken and complexes extracted with 1, 2, 3 and 4 ml chloroform by vigorous shaking, the extracts were then collected in a 10 ml volumetric flask and made up to 10 mL with chloroform.

1 mL each of 0.5, 1, 10, 40 and 100 µg/mL Atropine standard solution was transferred to different separating funnels. Then 5 ml of pH 4.7 phosphate buffer and 5 ml of BCG solution was taken and the mixture was shaken with extracts with 1, 2, 3, and 4 ml of chloroform. The extracts were then collected in 10 ml volumetric flask and then diluted to adjust solution with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against the blank prepared as above but without Atropine. The concentration of TA compound in the VALEs was determined using an equation $y = 0.0025x + 0.029$ and $R^2 = 0.9324$ obtained from a standard atropine graph. The total concentration of alkaloid was expressed in µg of atropine equivalent per mg dry sample weight.

All extraction procedures were performed in triplicate (n = 3) for each vegetable sample

Study animals

Male and female Wistar albino rats (130 ± 20 g) were obtained from the Animal Research Facility at Mbarara University of Science and Technology (MUST). The animals were acclimatised for 7 days under standard laboratory conditions of 21 ± 3°C, 12/12-hour light/dark cycle and 56 ± 4% humidity [35]. During the period of acclimatisation, all rats received normal pellet diet and water *ad libidum*. This study was approved and registered by the MUST Research Ethics Committee (approval number, MUST-2021-52) and subsequently by Uganda National Council for Science and Technology (registration number, HS1840ES). The study is reported in accordance with ARRIVE guidelines.

Acute oral toxicity study of Hs, Vu, and Sn ALEs

Acute oral toxicity testing was performed using the Up-and-Down method stated in the Organization for Economic Cooperation Development (OECD) guidelines No. 425 [35]. Briefly, after acclimatisation of the animals, one female rat was randomly allocated to a group that received a vegetable extract. The rats were fasted for 6 hours before the oral administration (using a gastric gavage needle) of the vegetable extracts at a starting dose of 175 mg/kg/b.w. The animals were then observed individually, after dosing, for the first 30 minutes, at least once per hour for the next 4 hours, periodically during the first 24 hours,

and daily thereafter. The dose progression was according to the Statistical Program for Acute Oral Toxicity (AOT425statpgm) Version 1.

The animals were monitored for a total of 14 days for the long term possible effects on the skin, respiratory rate, salivation, diarrhea, lethargy, behavioral patterns and convulsions. Treated animals were compared with the control animals that received only distilled water.

Induction of T2DM using high fat diet/streptozotocin (HFD/STZ) rat model

Male rats were given a HFD (60% fat, 25% protein and 17% carbohydrate, as a percentage of total kcal *ad libitum*) for 2 weeks. The preparation of HFD was done according to [36], with the ingredient composition in Table 1. After dietary manipulation, the animals received multiple intraperitoneal injection (i.p) of low doses (40 mg/kg/b.w.) of STZ freshly dissolved in 0.1M Citrate buffer of 4.7 pH, twice a week [37, 38]. After 2 weeks of STZ injection, rats were tested for successful induction of T2DM. Those with a fasting blood glucose (FBG) level greater than 7 mmol/L were considered to have T2DM and were left for 4 days for the stabilisation of the blood sugars before enrolling them for the study.

Table 1
Composition of the high fat diet

Ingredients	Amount g/kg
Powdered NPD	365
Lard	310
Casein	250
Cholesterol	20
Mixture of vitamins and minerals	60
DL methionine	03
Yeast powder	01
Sodium chloride	01

Study of the hypoglycemic effect of Hs, Vu and Sn ALEs on HFD-STZ induced diabetic rats

The diabetic rats were randomly distributed into 9 groups of 5 rats each.

Group 1 (Diabetic control): Diabetic rats received distilled water.

Group 2 and 3 (Hs-100 and Hs-200): Diabetic rats received aqueous extract of Hs at a dose of 100 and 200 mg/kg/b.w.

Group 4 and 5 (Sn-100 and Sn-200): Diabetic rats received aqueous extract of Sn at a dose of 100 and 200 mg/kg/b.w.

Group 6 and 7 (Vu-100 and Vu-200): Diabetic rats received aqueous extract of Vu at a dose of 100 and 200 mg/kg/b.w.

Group 8 (Reference drug): Diabetic rats received 5 mg/kg/b.w. of glibenclamide

Group 9 (Normal control): Normal rats received distilled water.

The administration of the vegetable aqueous leaf extracts, distilled water and reference drug to the respective groups was done daily for 12 days. The FBG levels were measured using a glucometer by withdrawing blood from the tail tip, and animal body weights were taken using an electric compact scale. These measurements were taken on 6-hour fasted animals on days 0, 4, 8, and 12.

Euthanasia of the experimental animals after the study period

Euthanasia was done using the chemical method, that is, after the study, induction of death in the animals was done by inhalation of an overdose of halothane [39, 40]. Briefly, the experimental rats (6) were placed in a plastic chamber, and cotton balls soaked with halothane were dropped in the chamber at different corners, and covered. Lack of response to bright light and absence of respiratory movement (for about 30 minutes) was confirmation of death. The carcasses of the euthanized animals were disposed incinerated.

Statistical analysis

All the statistical analyses were performed using Graph Pad Prism (version 8) software. Results were expressed as Mean $\pm$ SD ($n = 5$ for animal studies and phytochemical extraction procedures were performed in triplicate ($n = 3$) for each vegetable sample). One-way analysis of variance (ANOVA) followed by Tukey's Multiple comparisons test was used to determine if there were statistically significant differences in the means of the FBG levels, body weight of the animals and the phytochemical quantities in the vegetables. Differences in mean values with $p < 0.05$ were considered to be statistically significant.

Results

Phytochemical screening of aqueous and methanol leaf extracts of Hs, Vu and Sn

Phytochemical screening showed that more compounds were present in aqueous than methanol solvent (Table 2). The phenolic compounds were present in all the vegetables although tannins were strongly present in the methanolic extract of Hs. Flavonoids were also strongly present in Sn aqueous extract whereas all the vegetables contained just traces of glycosides and alkaloids.

Table 2

Phytochemical screening of aqueous and methanol leaf extracts of Hs, Vu and Sn vegetables

Phytochemical	Vu		Hs		Sn	
	Aqueous extract	Methanolic extract	Aqueous extract	Methanolic extract	Aqueous extract	Methanolic extract
Glycosides	-	-	-	+	-	+
Phenols	++	++	++	+++	+	++
Tannins	-	+	-	+++	-	-
Flavonoids	-	+	++	+	+++	-
Saponnins	++	-	+	-	-	-
Steroids	+	-	+	+	+	-
Terpenes	+	-	-	+	+	-
Coumarins	++	-	+	++	-	+
Alkaloids	-	-	-	+	+	-
+++ : strongly present, ++ : present, + : weakly present, - : absent						

Phytochemical quantification of aqueous leaf extracts of Hs, Vu and Sn vegetables

In this study, the phytochemical compounds in the VALEs were quantified and the results obtained are shown in Table 3. These phytochemical quantities vary significantly at $P < 0.05$. Hs contained significantly highest TP and least amount of TS compounds. TF, TT and TA were all significantly highest in Sn. However, Vu contained significantly highest TS and significantly least quantify of TA.

Table 3

Phytochemical quantification of aqueous leaf extracts of Hs, Vu and Sn vegetables

Vegetables	Mean $\pm$ SD (μg standard equivalent/mg vegetable dry weight)				
	Total Phenolic	Total Flavonoids	Total Tannins	Total Saponins	Total Alkaloids
<i>Solanum nigrum</i>	50.04 $\pm$ 0.19 ^c	71.35 $\pm$ 0.14^a	172.17 $\pm$ 8.69^a	81.46 $\pm$ 0.24 ^b	119.71 $\pm$ 3.64^a
<i>Vigna unguiculata</i>	63.59 $\pm$ 0.10 ^b	65.79 $\pm$ 0.08 ^b	102.85 $\pm$ 16.93 ^b	125.78 $\pm$ 0.47^a	44.64 $\pm$ 1.69 ^c
<i>Hibiscus sabdariffa</i>	66.11 $\pm$ 0.19^a	49.00 $\pm$ 0.08 ^c	112.49 $\pm$ 7.53 ^b	40.2 $\pm$ 0.00 ^c	67.41 $\pm$ 3.08 ^b

Means that do not share a letter are significantly different, Significance level of $p < 0.05$.

Acute Oral toxicity study of the aqueous leaf extracts of Hs, Sn and Vu vegetables

The oral limit doses of 175, 550, 1750 and 5000 mg/kg/b.w. (recommended by the AOT425statpgm software) of aqueous extracts of Hs, Sn and Vu vegetables, caused no death in the rats. No toxicity signs were observed throughout the short and long term period of observation. Therefore, the aqueous leaf extracts of these vegetables may be orally safe at these dose limits and the oral LD_{50} may be considered to be greater than 5000 mg/kg/b.w. in Wistar rats.

Induction of T2DM using HFD-STZ in Wistar rats

After dietary manipulation with HFD for 2 weeks, there were increases in animal body weights. By the end of the second week of STZ injection, high water intake, frequent urination and a decrease of animal body weight was observed. The FBG levels greater than 11 mmol/L were recorded and during the 4 days of blood glucose stabilization, all these signs of T2DM persisted.

Hypoglycaemic effect of aqueous leaf extracts of Hs, Sn and Vu vegetables on FBG level of HFD-STZ induced diabetic rats

Among the different vegetable extract treatments, the Hs extract showed the highest significant hypoglycemic activity (54% and $p < 0.0002$) onset (4th day) at a dose of 100 mg/kg/b.w. However, at the end of the 12 days of study, its dose of 200 mg/kg/b.w. had reduced the FBG levels (at the same level of significance) by 65%, which was much higher than Sn (57% and $p < 0.0002$) and Vu (43% and $p < 0.002$) extracts. The activity of Hs and Sn extracts was observed to be dose-dependent. The Vu extract showed a delayed onset of a significant hypoglycemic activity (32% and $p > 0.03$ on day 8), although the hypoglycemia significantly increased by day 12 to 43% and $p < 0.002$ at 100 mg/kg/b.w. dose. Glibenclamide used as a reference standard drug in this study, showed the least hypoglycemic activity at the beginning (4th day); however, it increased most significantly to 66% and $p < 0.0001$ by the end of the study. The vegetable extracts and drug hypoglycemic activities were compared to the diabetic control. These results are shown in Table 4a.

Table 4

a: Hypoglycemic effect of aqueous leaf extracts on Hs, Sn and Vu vegetables on FBG level of HFD-STZ induced diabetic rats

Treatment groups	Mean FBG level $\pm$ SD (mmol/L)				
	Dose (mg/kg)	Day 0	Day4	Day 8	Day 12
Normal control	-	5.1 $\pm$ 1.1	4.7 $\pm$ 0.5	5.2 $\pm$ 0.5	5.5 $\pm$ 0.7
Diabetic control	-	16.1 $\pm$ 6.6	23.7 $\pm$ 2.9 ^a	22.3 $\pm$ 4 ^a	26.8 $\pm$ 2.9 ^a
Glibenclamide	5	18.7 $\pm$ 5.4	17.3 $\pm$ 6 ^{*,b} (27%)	11.7 $\pm$ 2.9 ^{**,b} (48%)	9.2 $\pm$ 1.5 ^{****,b} (66%)
<i>Solanum nigrum</i> (Sn)	100	14.6 $\pm$ 6	16.1 $\pm$ 1 ^{**,b} (32%)	16.1 $\pm$ 13 ^{**,b} (28%)	15.3 $\pm$ 3.4 ^{****,b} (43%)
	200	19.4 $\pm$ 1.8	15.4 $\pm$ 2.5 ^{**,b} (35%)	14.1 $\pm$ 1.3 ^{***,b} (37%)	11.4 $\pm$ 2.6 ^{****,b} (57%)
<i>Hibiscus sabdariffa</i> (Hs)	100	22.5 $\pm$ 4.8	10.9 $\pm$ 3.3 ^{***,b} (54%)	15.6 $\pm$ 4.7	10.3 $\pm$ 2 ^{***,b} (62%)
	200	12.4 $\pm$ 5.7	13.4 $\pm$ 4.2 ^{**,b} (43%)	11.9 $\pm$ 4.8 ^{**,b} (47%)	9.3 $\pm$ 1.3 ^{***,b} (65%)
<i>Vigna unguiculata</i> (Vu)	100	19.1 $\pm$ 3.4	16.4 $\pm$ 5.3	14 $\pm$ 5.6 ^{*,b} (32%)	15.2 $\pm$ 5.1 ^{**,b} (43%)
	200	22.3 $\pm$ 7.1	20.6 $\pm$ 8	21.6 $\pm$ 7.2	21.4 $\pm$ 7.1

Results are expressed in Mean $\pm$ SD (Standard Deviation), n = 5. *p < 0.03, **p < 0.002, ***p < 0.0002 and ****p < 0.0001. ^ap < 0.05 in comparison with Normal control and ^bp < 0.05 in comparison with the diabetic control group.

Effect of ALEs of Hs, Sn and Vu on animal body weight of HFD-STZ induced diabetic rats.

There was generally insignificant decrease (p > 0.05) of animal body weight in the treatment groups (compared to the diabetic group) during the study period. Body weight of the animal group that received Vu extract at 200 mg/kg dose insignificantly increased (p > 0.05) most compared to the rest of the animal groups. The animal group that received Hs-200 mg/kg dose showed an insignificant (p > 0.05) reduction in body weight. Glibenclamide did not reduce the animal body weights in this study (Table 4b).

Table 4

b: Effect of aqueous leaf extracts of Hs, Sn and Vu vegetables on animal body weight of HFD-STZ induced diabetic rats. There was no significant change in body weights across the twelve days of experimentation.

Test sample	Mean Body Weight $\pm$ SD (Kg)				
	Dose mg/kg	Day 0	Day 4	Day 8	Day 12
Normal control	-	203 $\pm$ 10.8	203 $\pm$ 11	205.1 $\pm$ 10.1	207.4 $\pm$ 10.2
Diabetic control	-	190.1 $\pm$ 27.4	190.6 $\pm$ 28	191.2 $\pm$ 26.4	186.7 $\pm$ 26.7
Glibenclamide	5	167.1 $\pm$ 28.1	186.4 $\pm$ 26	188.7 $\pm$ 26.6	186.1 $\pm$ 26.4
<i>Solanum nigrum</i>	100	192.5 $\pm$ 13	191.3 $\pm$ 15.4	187.9 $\pm$ 16.6	186.5 $\pm$ 16.8
	200	211.1 $\pm$ 15.1	201 $\pm$ 21	200.1 $\pm$ 20.1	199 $\pm$ 20.1
<i>Hibiscus sabdariffa</i>	100	212.5 $\pm$ 26.2	220.4 $\pm$ 23.9	203 $\pm$ 27.6	201 $\pm$ 27.5
	200	172.1 $\pm$ 40.6	178 $\pm$ 46.3	165 $\pm$ 41.3	163.3 $\pm$ 41.9
<i>Vigna unguiculata</i>	100	212.6 $\pm$ 27.3	208.2 $\pm$ 28.7	206.2 $\pm$ 28.5	204.9 $\pm$ 29.1
	200	230.7 $\pm$ 42.3	229.2 $\pm$ 42.5	226.6 $\pm$ 42.1	225.5 $\pm$ 42.3

Discussion

As aforementioned, the prevalence of hyperglycaemia related to T2DM is varied depending on location, age and sex. This is similar in Uganda where it was found to be highest in Ankole and lowest in Teso sub-region, and this variance was dependent on predisposing factors like diet among others [41]. This study was designed to screen and quantify the phytochemicals and to investigate the hypoglycemic property of the most frequently eaten vegetables in these two sub-regions in an attempt to subsequently explain the variance in national regional prevalence. These vegetables were chosen because they are an important aspect of traditional diets [42], in addition to being recommended for a healthy diet [43], and of which diet in itself is a component of an environmental (apart from genetics) basis of diabetes. In this regard, scientific attention through research in the last decades and in different parts of the world has shown that plant based diets and therapies have potential to prevent diabetes, and manage or even treat its complications like hyperglycemia [44, 45]. The vegetables Hs, Vu and Sn are being used in many African countries as traditional plant food and remedies for alleviating hyperglycaemia, but the species in

Uganda have not been scientifically evaluated for that effect. The combination of HFD-STZ is used for inducing T2DM in animals because HFD causes intra-cellular fat that blocks the insulin receptor sites hence causing insulin resistance [46]. Streptozotocin (in multiple low doses) is well known for causing pancreatic islet cell toxicity which consequently leads to partial impairment in insulin secretion [37]. Continuing impairment of insulin secretion and its resistance in the target tissues will exacerbate carbohydrate, protein and fat metabolic disturbances and leads primarily to hyperglycemia [24].

Phytochemical screening was performed and water solvent extracted more components than methanol because it is a universal solvent [47]. Phenolic and flavonoid compounds were found in all the vegetables leaves; however, tannins and alkaloids were in trace amounts. When these phytochemicals were quantified, their quantities varied significantly among the vegetables.

Hs was found to contain significantly highest quantity of TP compared to Vu and Sn. Chlorogenic acid is one of the phenolic acids reported in the leaves of Hs [48]. Protocatechuic acid is another phenolic compound in Hs extract though its higher in the calyces [49]. Sn contained the significantly highest amount (71.35 µg equivalence/mg dry weight) of TF compounds although the amount is lower (33.83µg equivalence/mg of dry weight) in Sadique et al's study of the same plant part [50].

Tannins and saponins are considered anti-nutritive because they interfere with the absorption of iron and impart bitterness to food respectively. The bitter taste of saponins can reduce their palatability since they are throat-irritating [51, 52]. Since the tender vegetables leaves are eaten cooked, heat inactivates these anti-nutritive compounds which explains the lack of reports of toxicity [13]. However, the traces of tannins and saponins found therefore means that these vegetables need to be boiled before eating to inactivate them, and therefore should not be eaten raw (as salad) [53]. This is in agreement with other studies, which showed that the Sn fruits/berries, calyces and seeds contain more or less of the phytochemicals found in the leaves [54–56].

These vegetable ALEs can be safe orally (in animals) even above the dose of 5000 mg/kg.b.w. Several studies on other parts of these vegetables including seeds, fruits and flowers, extracted with other solvents like methanol have shown similar safety profiles [57–59]. Ethnobotanical literature indicates that these leafy vegetables have been eaten as part of diets for centuries without any toxicity reports [60, 61]. Though Sn leaves are considered edible, they contain a glycol-alkaloid called solanine which is reported to be toxic in mature leaves, and more toxic in unripe berries compared to the ripe ones [62]; however, simple boiling of the young leaves is enough to inactivate the alkaloid [63].

Effect of the various treatments on the FBG levels and body weights of the HFD-STZ induced diabetic rats

The vegetables Hs, Sn and Vu are all used in African communities and Vu is especially considered a human vegetable because its leaves and seeds have been eaten for centuries particularly in the tropics for its high protein, carbohydrates and vitamin content [61, 64]. The calyces of Hs have been exploited for

beverage and dyeing purposes compared to the leaves which have been used more for soup and less for drinks [55, 65]. The leaves of Sn have been eaten for sauce more than its ripe berries [56, 66].

During our study, these vegetable ALEs insignificantly reduced body weight of the animals compared to the diabetic control. However, the contrary has been reported by other researchers who studied different vegetable parts extracted with different solvents. This disagreement in the results could be due to several reasons, like the period of observation which could have been insufficient for the significant effect from the extracts' metabolic activity. It is also possible that environmental factors such as soils and climate can have an influence on the phytochemical quality and quantity of the vegetable species [67, 68]. The solvent used for extraction from the plant part can also have an effect on the quality and quantity of the phytochemicals extracted (Pandey and Tripathi, 2014; Raya et al, 2015; Stephane et al., 2021) Amaya et al. and Ojulari et al. (2019) studied the aqueous calyx extract of Hs and reported a significant reduction of the animal body weight [69, 70]. Nderitu et al. (2023) and Aabideen et al. (2022) reported Sn aqueous and other solvent extracts to be able to significantly reduce animal body weight [71, 72]. Vu too was observed to have a similar effect in a significant manner in other various studies [57, 73]. This animal body weight decrease has been attributed to the vegetable polyphenolic compounds (flavonoids) that may suppress growth of the adipose tissue through their anti-angiogenic activity and by modulating adipocyte metabolism [74, 75].

The FBG levels of the animals were significantly reduced in comparison to the diabetic control after Hs, Sn and Vu ALE administration, and this result is in agreement with other studies on different solvent extracts and vegetable parts. The calyces of Hs have been exploited for beverages and the scientific validation on animal models shows a hypoglycemic property [76–78], however, there is scarce literature of the same effect by the leaves in this species. The same effect was also observed by the leaves and seeds of Vu [19, 54], and likewise by the leaves and berries of Sn [58, 79].

These vegetables do contain polyphenolic compounds (for example phenolic acids and flavonoids) [45, 80, 81] that have been reported to exert influence on homeostasis of blood glucose level by different mechanisms. For example, they can inhibit the intestinal digestive enzymes, delaying the digestion of complex sugars into simple sugars, and thus reducing the absorption of the latter, and consequently lowering the blood glucose level [82, 83]. This mechanism of action was reported in Hs fruits extracted by methanol solvent in a study by Raheem et al.[84]. The mechanism is similar to that of the synthetic intestinal enzyme inhibitor drugs but with less likelihood of side effects such as abdominal gas and diarrhea [45]. These polyphenolic compounds have further shown a significant influence on the up-regulation of the expression levels of glucose transporter 4 (GLUT4) genes in cell lines, which results in higher glucose uptake in similar levels to that taken by insulin. This was demonstrated by Boue et al. (2016) in two pigmented rice bran extracts [85].

A protein with similar molecular mass and amino acid sequence with that of bovine insulin has been isolated from the empty pods and seed coats of Vu [86], *Spinacia oleracea* (spinach) [87] and fruit of

Momordica charantia (bitter gourd) [88]. This insulin-like protein is reported to be in most green leafy vegetables though it is not proven to be in any of these studied species.

The dose dependence of these vegetables' hypoglycemic activity was also recorded by other authors that studied other reputed hypoglycemic vegetables like *Vernonia amygdalina* (bitter leaf) [89], *Basella alba* (vine spinach) [90] and *Amaranthus spp* (amaranth) [91] though extracted in different solvents. This could be due to the compounding effect of the extract administration.

Glibenclamide is a cardinal drug (second generation sulfonylurea compound) used in the treatment of T2DM and it was used as a control in this study. It did reduce the FBG levels better than the vegetable extracts at the end of the study although it had the lowest activity at the start. Detrimental effect of hyperglycemia on its absorption in the intestines can explain its low activity at the start of this study. However, it does have beta cell and extra-pancreatic effects like those on skeletal and adipose tissue when finally absorbed in the blood stream. In the pancreas, it works by inhibiting the potassium ATP channels, which cause membrane depolarization, calcium influx and finally activation of the insulin secretory machinery [92, 93]. In the muscles and adipose tissue, it works through increased synthesis of the glucose transporter 1 (GLUT 1) [94, 95], thereby explaining the achievement of the highest reported hypoglycemic activity in this study.

Conclusion

The results from this study show that these vegetable ALEs (especially for Hs and Sn) can intervene in hyperglycemia and they can serve to help sustain glycemic control in the long term and prevent the development of complications. Their safety profile supports their consumption in the diet, as alternatives and/or dietary complements. Moreover, concomitant use with synthetic anti-hyperglycemic therapies is encouraged to make up for the down side of synthetic drugs like accessibility, high costs and side effects [96, 97]. There is however need for more research on drug-phytochemical interaction to avoid experiencing the opposite effects [98]. We do recommend more studies on different doses and extracts and covering longer periods of observation, in order to reach a clear-cut conclusion. Research on the exact phenolic compound(s) responsible for the hypoglycemic effect of these vegetable species is necessary to advance knowledge on their mechanistic effects with a view to pharmaceutical product development.

Study limitations

One of the limitations of the current study was that we used only leaves from each of the studied plant species as starting material and did not study other plant aerial parts or roots which probably contain different amounts of the active phytochemicals. In addition, we did not consider the different growth stages of the leaves at the time of collection. The factors mentioned have the potential to influence the quality and quantity of the phytochemical compounds extracted, and hence affect the outcomes of the study. A third limitation of our study pertains to the unexplored role of other factors such as assessing

physical activity, which can lower high blood glucose. Given the experimental nature of our study, however, overall, the data in the present study indicate that the aqueous leaf extracts possess hypoglycemic effects and can be considered effective in prevention and management of hyperglycemia. Further studies are warranted to explore the effects of the identified phytochemicals and the role of exercise combined with consumption of these vegetable parts in lowering elevated blood glucose.

Abbreviations

ANOVA	Analysis of Variance
OECD	Organization for Economic Co-operation and Development
T2DM	Type 2 Diabetes Mellitus
WHO	World Health Organisation
Hs	<i>Hibiscus sabdariffa</i>
Vu	<i>Vigna unguiculata</i>
Sn	<i>Solanum nigrium</i>
ALEs	Aqueous Leaf Extracts
HFD-STZ	High Fat Diet-Streptozotocin
FBG	Fasting Blood Glucose
IDF	International Diabetes Federation
NPD	Normal Pellet Diet
i.p	Intra-peritoneal
GLUT 1	Glucose transporter 1
GLUT 2	Glucose transporter 2
GLUT 4	Glucose transporter 4
GAE	Gallic acid equivalent
QEF	Quercetin equivalent of flavonoids
TP	Total phenolics
TF	Total flavonoids
TT	Total Tannins
TS	Total Saponins
TA	Total Alkaloids

Declarations

Ethics approval and consent to participate

The study was approved by Mbarara University of Science and Technology Research Ethics Committee (MUST-REC) under Protocol number MUST-2021-52 and registered with the Uganda National Council for Science and Technology (UNCST) under registration number HS1840ES. The study is reported in accordance with ARRIVE guidelines.

Consent for publication

Not applicable

Competing interests

The authors declare no conflict of interest.

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Author Contribution

P.E.O., C.A, P.E.A. and E.A.O. contributed to the conception of the study, C.A, P.E.A. and E.A.O. contributed to the design of the work, C.A. collected and analyzed the data, C.A., P.E.A., and E.A.O. contributed to the interpretation and drafting of the work, P.E.A and E.A.O. substantively revised it, and all authors have reviewed the final manuscript and approved the submitted version (and any substantially modified version that involves the author's contribution to the study). All authors have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. Currently they are with the corresponding author

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