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Research Article

Antihyperglycaemic Constituents from Cleistopholis Patens and Sansevieria Liberica as Justification of their Antidiabetic Ethnomedicinal Claims

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Abstract

The antihyperglycaemic activity of the methanol leaf and rhizome extracts of *Cleistopholis patens* and *Sanseviera liberica*, respectively has been reported and their dichloromethane and n-hexane partitioned fractions, respectively were found to be active. This study therefore evaluated the antihyperglycaemic activity of the chromatographic column fractions of the dichloromethane and n- hexane partitioned fractions of *Cleistopholis patens* and *Sanseviera liberica*, respectively. The glucose lowering constituents of the active subfractions were also isolated thereby further justifying their antidiabetic ethnomedicinal usage in Nigeria. The active dichloromethane and n-hexane fractions of methanol leaf and rhizome extracts of *Cleistopholis patens* and *Sanseviera liberica*, respectively were subjected to column chromatography. The subfractions obtained were assayed for antihyperglycaemic effect using glucose-induced hyperglycaemic model with glibenclamide (5 mg/kg) and 1 % Tween 80 in normal saline as positive and negative controls, respectively. The most active column fractions of the two plants were further purified by various chromatographic separations which led to the isolation of their antihyperglycaemic constituents. The results of the study showed that subfractions C_2, C_4, C_5, and C_8 of *C. patens* at 400 mg/kg gave comparable antihyperglyceamic activity to glibenclamide (5 mg/kg). Also, subfractions C_2, C_4, C_8 and C_9 of the n-hexane fraction of *S. liberica* were comparable in activity and gave comparable and similar profile of activity to glibenclamide (5 mg/kg). The structural elucidation of the isolated β-sitosterol from *C. patens* and β-stigmasterol from *S. liberica* that were carried out using 1D and 2D NMR spectra and their NMR data compared well with literature. The study concluded that the β- sitosterol from *Cleistopholis patens* and β-stigmasterol from *Sanseviera liberica* were some of the antihyperglycaemic constituents of the plants and thus justified their folkloric antidiabetic usage in Nigeria.

Keywords : Antihyperglycaemia, β-sitosterol, β-stigmasterol, Diabetes mellitus, medicinal plants.

Introduction

Diabetes mellitus is a non-communicable disease characterized by deficiencies in the metabolism of fats, carbohydrates and protein that result in persistent hyperglycaemia due to insulin deficiency or inadequate insulin secretion [1,2]. Global epidemiology showed that about 422 million people are diabetic with the majority of the patients living in low- and middle-income countries. Type 1 and type 2 diabetes mellitus contribute 5-10 % and 90-95 %, respectively to the population of diabetic subjects worldwide [3] while over 1.5 million deaths occur because of diabetes every year [4]. It is presented with excess weight loss, stroke, blindness, and other severe complications [5]. Over the years, various

kinds of synthetic hypoglycaemic drugs have been developed for the management of diabetes. However, the drugs have been variously reported to have significant deficiencies such as, serious side effects and lack of potency in diabetic subjects [6,7]. Hence, increased focus on exploring plants and other natural sources of antidiabetic agents is important.

Cleistopholis patens (Benth.) Engl. & Diels (Annonaceae) also called 'Apako' and 'Ojo' among the Yorubas and Igbos in Nigeria, respectively, is a tree reaching to 30 m high, occurring from Sierra Leone to Uganda and Zaire [8]. Ethnomedicinally, it is used in the treatment of jaundice, infective hepatitis, stomach disorders as well as in the management of diabetes [9-12]. It has been reported for antiplasmodial, insecticidal and anthelminthic activities [13,14]. Some isolated compounds from the plant include β-stigmasterol, β-sitosterol, campesterol which are terpenoids [15]. Others are β-hydroxysampangine, bornyl-*p-trans*-coumarate and bornyl-p-cis-coumarate, α-copaene, δ-cadinene, β- caryophyllene [13,14]. In 2017, Ayoola and his coworkers reported the antihyperglycaemic and antioxidant activities of the extract of *Cleistopholis patens* and its most active dichloromethane partitioned fractions [12]. However, the compounds responsible for its glucose lowering effect were not identified.

Sansevieria liberica Gerome and Labroy (Agavaceae) is known as ''Ida orisa'' in the Western Nigeria and is folklorically used for the treatment of asthma, diabetes, abdominal pains, hypertension, menorrhagia, piles, sexual weakness [16,17]. Antihypertensive, anticancer, diuretic, antioxidant; hepatoprotective, hypoglycaemic and antihyperglycaemic activities have been reported [18-23]. Some isolated compounds from its stem bark and leaf include, pavetannin, aplysamine-2, abscisic acid, α-conidendinin and quercetin-3-O-α-L-arabinofuranoside [24]. Terpenoids such as β-stigmasterol, β- sitosterol, campesterol have also been isolated from the plant [18]. In a recent study, Ayoola and his team reported a significant and consistent antihyperglycaemic and antioxidant activities of the extract, partition and column fractions of its rhizome [23]. The n-hexane fraction of the extract was found to give a promising and comparable activity with glibenclamide, the positive control [23]. Similar to that of *C. patens*, the compounds responsible for the antihyperglycaemic activity of *S. liberica* are yet to be identified. This study was therefore designed to further purify the active dichloromethane and n-hexane partition fractions of *C. patens* and *S. liberica*, respectively with the aim of isolating the antihyperglycaemic constituent(s) of the two plants with a view to further justifying their antidiabetic ethnomedicinal claims.

Materials and Methods

Chemicals, Equipment and Instrumentation

UV Spectrophotometer (Model M107, SpectronicCamspec Ltd, U.K.), Accu-chek ™ Glucometer (model GB

11558973, Roche, Germany) with Accu-chek ™ test strips (Roche, Germany), column chromatography

(Dimension: 60 × 4 cm, silica gel mesh 70–230) apparatuses were used. Others were aluminium plated thin-layer chromatographic (silica gel 60 F254, 0.25 mm) and glass plated preparative thin-layer chromatographic (silica gel 60 F254, 0.25, 0.5, 1, 2 mm, Whatman Inc., U.S.A.), silica gel (70-230 mesh, Merck & Co., Inc., U.S.A.). Nuclear magnetic resonance (NMR) spectra (400, 500, and 600 MHz) were obtained with Bruker AMX 400, Varian Nova 500, and Varian Unity Plus 600 NMR instruments. All solvents used were of analytical grade.

Animals

Healthy male and female albino Wistar rats (120-180 g) bred under standard conditions (temp. 27 ± 3 °C, relative humidity 65%) at the animal house, Department of Pharmacology, Faculty of Pharmacy, O.A.U., Ile- Ife, Nigeria were used for the study. They were fed on a standard commercial rat pellet diet (Bendel Feeds, Nigeria) and water was given as required. Groups of five rats were fasted for 18 h before administration of glucose, column fractions, drug or vehicle [12,23]. All animal experiments conformed to the *Guide for the Care and Use of Laboratory Animals* published by the National Academies Press (Committee for the update of the guide for the care and use of laboratory animals) [25].

Plant Materials, Extraction and Partition Fractions

Cleistopholis patens leaf and *Sansevieria liberica* rhizome were separately collected from the medicinal plant garden, Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University (OAU), Ile-Ife, Nigeria. They were identified, authenticated and voucher specimens, IFE 16472 and FPI 2176, respectively were deposited at the Pharmacy Herbarium, Department of Pharmacognosy, Faculty of Pharmacy and IFE Herbarium, Department of Botany, O.A.U, Ile-Ife. *Cleistopholis patens* leaf were air-dried, powdered and extracted with methanol at room temperature, filtered and concentrated *in-vacuo* to give 10.8 % w/w yield. The methanol extract was suspended in water, successively partitioned with n-hexane and ethylacetate to obtain their corresponding n-hexane, ethylacetate and aqueous partition fractions that were concentrated *in-vacuo*. The rhizome of *Sansevieria liberica* was washed with water, chopped into small pieces, oven-dried at 60 °C, powdered and 4 kg of the powdered material was extracted with

methanol at room temperature and concentrated *in-vacuo* to give 11.0 % w/w yield. The methanol extract was suspended in water, successively partitioned with n-hexane and ethyl acetate and concentrated *in vacuo* to obtain their corresponding fractions.

Column chromatography (CC) of *C. patens and S. liberica* fractions

The active antihyperglycaemic dichloromethane fraction (24 g) of the methanol leaf extract of *C. patens* was adsorbed on silica gel and subjected to column chromatography using gradient solvent systems of increasing polarity comprising of n-hexane, dichloromethane, ethylacetate and methanol. The collected eluates were bulked into subfractions **CP_1-CP_{15}** based on their TLC profile. Similarly, n-hexane fraction (15.0 g) of the methanol rhizome of *S. liberica* extract was subjected to column chromatography using gradient solvent systems of increasing polarity of n-hexane, dichloromethane, ethylacetate and methanol. The resulting eluates were bulked into **CP_1-CP_9** based on TLC analysis.

Antihyperglycaemic Assay of Column Fractions

Overnight fasted normal rats were orally administered with glucose (10 g/kg) and those with blood glucose level ≥ 7.0 mmol/L (126 mg/dL) after 0.5 h (T_0) were considered hyperglycaemic which were selected and divided into groups of five rats. Each group of rats was separately orally administered with 1% Tween 80 in normal saline (negative control), column fractions and glibenclamide at 5 mg/kg (positive control). A drop of blood was taken from the tip of the tail of each rat at 0.0, 0.5, 1.0, 2.0 and 4.0 h and the glucose level were measured using a glucometer and strip. The blood glucose levels at 0.0 h (T_0) were recorded as 100 % while the others were expressed as percentage of the T_0 values [26-29].

Isolation of Compound 1 from the Dichloromethane Fraction of *C. patens*

Subfraction **CP_4** (2.0 g) with comparable antihyperglycaemic activity with glibenclamide and fewer TLC spots was adsorbed on silica gel, subjected to CC, eluted with gradient mixtures of solvents and the resulting fractions were bulked by TLC into 20 bulked subfractions coded **CP_4A- CP_4T**. Subfraction **CP_4G** (39.0 mg) was subjected to PTLC (0.5 mm, $CHCl_3$: MEOH; 1:1). The resulting bands gave rise to crystalline white powder, **A** (12 mg), **B** (2.0 mg) and **C** (19.0 mg). Isolate A was adjudged pure based on its TLC profile and labelled as compound **1**.

Isolation of Compound 2 from the Active n-Hexane Fraction of *S. liberica*

Antihyperglycaemic CC fraction, **C_4** (0.65 g) of S. liberica was adsorbed on silica gel, eluted with solvent systems and the resulting gradient fractions were bulked into **D_1-D_{10}** based on their TLC profile. Subfraction **D_5** (0.15g) was further chromatographed on a silica gel, gradiently eluted with solvent systems and bulked into seven subfractions, **E_1-E_7** monitored by TLC. Subfractions **E_3** and **E_4** were purified by PTLC (0.5 mm) (N-hex: EtOAc; 9:1) and (N-hex: EtOAc; 8.5:1.5), respectively to yield compounds **B** (20.0 mg) and **C** (18 mg), respectively.

Identification of the isolates

$^1H/^1H$-NMR, Homonuclear Correlated Spectroscopy (COSY), $^1H/^{13}C$-NMR- Heteronuclear Multiple Bond Correlation (HMBC), Heteronuclear Single Quantum Correlation (HSQC), Total Correlation Spectroscopy (TOCSY), and Electro-Spray Ionisation Mass Spectrometry (ESIMS) data of the isolate A from *Cleistopholis patens* were compared with information in the literature and its identity was confirmed to be, β-sitosterol, while isolates **B** and **C** from Sansevieria *liberica* were one and the same compound and labelled as compound 2 which was found to be, β-stigmasterol [30-32] (Figure 1).

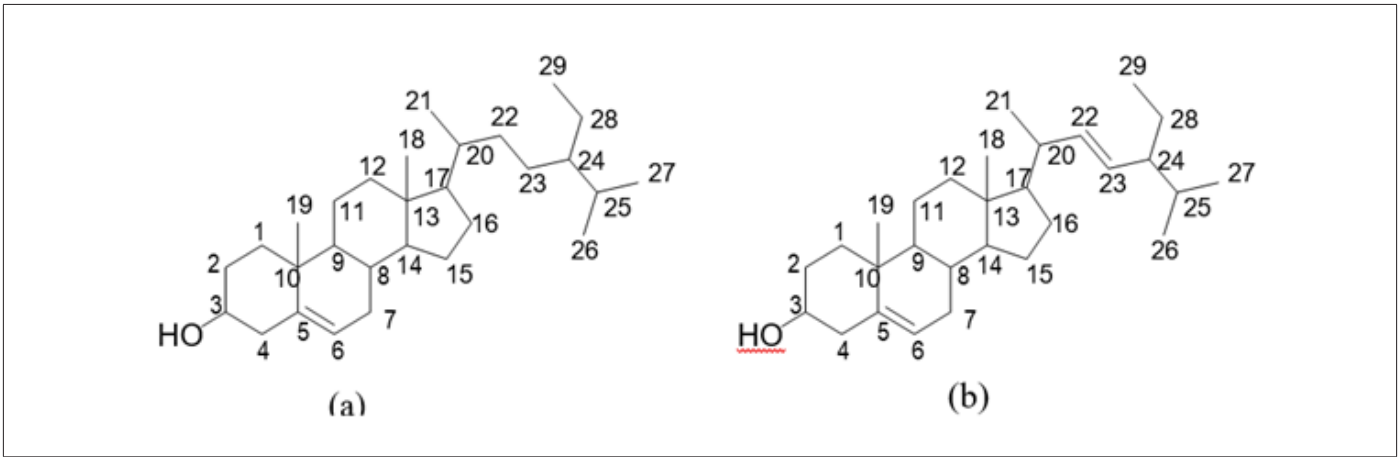

Figure 1: Structures of compounds 1 and 2

Statistical Analysis

The results obtained from the study were expressed as the mean ± SEM for the number (n=6) of animals in the groups. One Way Analysis of Variance (ANOVA) was used followed by Bonferroni t-test or Student Newman-Keuls post hoc tests to determine the source of significant differences. ($p<0.05$) was taken as statistically significant.

Results and Discussion

(Table 1) Data show the mean ± SEM blood glucose levels at the different time points expressed as percentage of levels at 0 h (T_0), n = 5. Values with different superscripts within columns are significantly different ($p<0.05$) while values with similar superscript are comparable ($p>0.05$). GLU (10 g/kg): glucose with 1% Tween 80 in normal saline (negative control); **C_1–C_{15}**: Bulked column fractions of n-hexane fraction of *C. patens*; **GLI:** Glibenclamide (positive control).

Table 1: Antihyperglycaemic Effects of the Dichloromethane Column Fractions of *C. patens*

Dose of fractions/ drug	Blood glucose levels as percentages of T0 (% percentage reduction in blood glucose relative to negative control at Tt)				
(mg/kg)	0 h	0.5 h	1 h	2 h	4 h
Glu (10)	100	83.79±3.81^{b,c}	85.89±0.50^{c}	76.45±1.71^{b,c}	74.18 ±1.97^{d,e}
C1	100	87.32±1.47^{b,c} (-4.21 %)	70.15±3.04^{a,b} (-18.33%)	64.77±2.93^{a,b} (-15.28%)	59.16±3.02^{c} (-20.25%)
C2	100	88.75±4.44^{b,c} (-5.92 %)	72.45±9.62^{a,b} (-15.65%)	51.69±2.14^{a} (-32.39%)	43.39±5.33^{a} (-41.51%)
C3	100	90.72±4.97^{b,c} (-8.27 %)	84.82±5.46^{b,c,d} (1.24%)	74.40±5.82^{b,c} (2.68%)	61.94±4.83^{c,d} (16.5%)
C4	100	64.14±4.79^{a} (23.45%)	59.90±4.33^{a} (30.26%)	57.68±4.73^{a,b} (24.55%)	38.88±6.69^{a,b} (47.59%)
C5	100	84.39±5.31^{b,c} (-0.72 %)	63.42±4.29^{a} (26.16%)	57.11±8.80^{a,b} (25.3%)	30.66±3.00^{a} (58.67%)
C6	100	87.14±3.61^{b,c} (-4.00 %)	72.85±3.06^{a,b,c} (15.18%)	55.62±6.27^{a,b} (27.25%)	42.77±6.29^{a,b} (21.41%)
C7	100	89.71±4.43^{b,c} (-7.07 %)	78.93±5.20^{b,c} (8.10%)	64.41±4.66^{a,b} (15.75%)	57.83±5.46^{c} (22.04%)
C8	100	84.86±1.84^{b,c} (-1.28 %)	68.71±1.46^{a,b} (20%)	57.71±0.11^{a,b} (24.51%)	46.62±1.6^{a,b,c} (37.15%)
C9	100	78.34±2.98^{b} (6.50%)	70.93±3.95^{a,b} (17.42%)	65.56±4.90^{a,b} (14.24%)	59.67±3.15^{c} (19.56%)
C10	100	78.10±4.47^{b,c} (6.79%)	71.22±4.41^{a,b} (17.08%)	64.84±2.81^{b} (15.19%)	55.85±2.80^{b,c} (24.71%)
C11	100	76.88±7.03^{a,b} (8.25%)	91.73±1.99^{d} (-6.80 %)	86.84±6.49^{c,d} (-13.60 %)	79.15±2.67^{e} (-6.70 %)
C12	100	71.10±2.51^{a} (15.15%)	68.14±3.83^{a,b} 20.66%	64.81±3.20^{a,b} (15.23%)	54.16±2.65^{b,c} (26.99%)
C13	100	79.35±3.79^{b} (5.30%)	96.58±1.86^{d} (-12.45 %)	85.93±3.27^{c,d} (-12.40 %)	78.52±2.41^{d} (-5.85 %)
C14	100	79.79±3.54^{b} (5.30%)	71.22±3.26^{a,b} (17.08%)	66.73±2.56^{a,b} (12.71%)	59.66±2.80^{c} (19.57%)
C15	100	92.84±1.90^{c} (-10.80 %)	91.17±3.78^{c} (-6.15 %)	89.76±4.36^{d} (-17.41 %)	92.35±6.01^{e} (-24.49 %)
GLI (5)	100	75.64±6.73^{a,b} (9.73%)	70.68±6.86^{a,b} (17.71%)	58.32±6.44^{a,b} (23.71%)	45.27±6.88^{a,b} (38.97%)

Glibenclamide, a sulphonylurea, that was used as the antidiabetic standard drug in this study has been reported to be working through minor early extra-pancreatic and major late insulin stimulation [33]. This has made it possible to understand the possible extra-pancreatic and insulin stimulating mechanisms of action of plant extracts/fractions/test agents in glucose-loaded and drug-induced hyperglycaemic rat's models. This happens when the test agents show similar activity profile to that of glibenclamide or other insulin stimulatory drugs as positive controls [34,35]. Also, it has been reported that results of the glucose loaded rats can be extrapolated to the type 2 diabetic state in humans [36]. Hence this model was employed in this study. The group of rats that received normal saline (negative control) showed a time dependent decrease in blood glucose level up to the fourth hour. This observation could be explained by the homeostatic regulatory mechanism in the normal animals, and it established the healthy state of the rats' pancreases [29,34,37]. The antihyperglycaemic activity demonstrated by the fifteen column fractions of the active dichloromethane partitioned fraction of *C. patens* in this work could be grouped into three. The first group consisted of subfractions $\mathbf{C_{11}}$, $\mathbf{C_{13}}$ and $\mathbf{C_{15}}$ that lacked activity from 0.5-4 h of the experiment which indicated that the antihyperglycaemic constituents of the chromatographic column fraction were not present in these subfractions. The second group were subfractions $\mathbf{C_1}$, $\mathbf{C_3}$, $\mathbf{C_6}$, $\mathbf{C_7}$, $\mathbf{C_9}$, $\mathbf{C_{10}}$, $\mathbf{C_{12}}$ and C14 that showed moderate and comparable ($p>0.05$) activity at 4 h of which only the activity of $\mathbf{C_3}$ and $\mathbf{C_7}$ was time dependent. They were however significantly ($p < 0.05$) less active than the positive control. Their moderate elicited antihyperglycaemic effect indicated that they contained some of the extra-pancreatic and insulin stimulating compounds in the plant. The third group, however, are subfractions $\mathbf{C_2}$, $\mathbf{C_4}$, $\mathbf{C_5}$, and $\mathbf{C_8}$ that gave high antihyperglyceamic activity that was comparable to glibenclamide (Table 1). This showed that the compounds responsible for the hyperglycaemia lowering activity were mostly concentrated in these subfractions. Similar profile of activity of subfractions $\mathbf{C_2}$, $\mathbf{C_5}$, and $\mathbf{C_8}$ with that of glibenclamide may indicate that their mechanism of action may be majorly through insulin stimulation [29,33,37]. Subfraction $\mathbf{CP_4}$ with comparable antihyperglycaemic activity with glibenclamide and fewer TLC spots was therefore chosen for further purification for the isolation of its antihyperglycaemic constituent (Table 2).

Table 2: Antihyperglycaemic effects of the n-hexane CC fractions of *S. liberica*.

Dose of fractions/ drug	Blood glucose levels as percentages of T0 (% percentage reduction in blood glucose relative to negative control at Tt)				
(mg/kg)	0 h	0.5 h	1 h	2 h	4 h
GLU (10)	100	83.79±3.81$^{a, b}$	85.89±0.50^{c}	76.45±1.71^{b}	74.18±1.97^{b}
C1	100	83.29±6.68^{a} (0.60%)	79.87±6.49$^{b, c}$ (7.01%)	75.49±7.62^{b} (1.26%)	71.01±7.28^{b} (4.27%)
C2	100	86.80±6.35$^{a, b}$ (-3.59%)	71.37±4.73$^{a, b}$ (16.90%)	52.95±4.27^{a} (30.74%)	39.58±3.53^{a} (46.64%)
C3	100	95.67±0.74^{b} (-14.18%)	84.66±2.38^{c} (1.43%)	76.13±5.01^{b} (0.42%)	74.47±2.64^{b} (0.39%)
C4	100	91.40±3.49^{b} (-9.08%)	70.98±2.78^{a} (17.36%)	55.22±3.06^{a} (27.77%)	39.28±1.90^{a} (47.05%)
C5	100	91.42±3.31^{b} (-9.11%)	86.06±2.34^{c} (-0.20%)	77.09±4.25^{b} (-0.84%)	68.13±4.52^{b} (8.16%)
C6	100	90.44±2.46^{b} (-7.94%)	86.93±2.92^{c} (-1.21%)	78.78±3.22^{b} (-3.05%)	70.01±4.01^{b} (5.62%)
C7	100	95.15±0.58^{b} (-13.56%)	91.88±0.76^{c} (-6.97%)	79.89±4.13^{b} (-4.50%)	67.78±4.22^{b} (8.63%)
C8	100	78.35±4.13^{a} (6.49%)	73.61±4.30$^{a, b}$ (14.3%)	55.86±3.50^{a} (26.93%)	33.06±2.11^{a} (55.43%)
C9	100	83.66±2.97$^{a, b}$ (0.16%)	64.99±2.09^{a} (24.33%)	46.35±1.6^{a} (39.37%)	36.69±0.93^{a} (50.54%)
GLI (5)	100	75.64±6.73^{a} (9.73%)	70.68±6.86$^{a, b}$ (17.71%)	58.32±6.44^{a} (23.71%)	45.27±6.88^{a} (38.97%)

Data show the mean ± SEM blood glucose levels at the different time points expressed as percentage of levels at 0 h (T0), n = 5. Values with different superscripts within columns are significantly different ($p<0.05$) while values with similar superscript are comparable ($p>0.05$). **GLU** (10 g/kg): glucose with 1% Tween 80 in normal saline (negative control); **C_1–C_9**: Bulked column fractions of n-hexane fraction of *S. liberica*; GLI: Glibenclamide (positive control).

The results of the antihyperglycaemic activity of the column fractions of the active n-hexane fraction of *S. liberica* showed that subfractions **C_1** and **C_3** were devoid of activity at 0.5-4 h which indicated that they lacked the antihyperglycaemic constituents of the partitioned fraction of the plant extract. Subfractions **C_5, C_6** and **C_7** lacked activity at 0.5-2 h but showed minor activity at 4 h which showed that they lacked the compounds for extrapancreatic activity with some insulin stimulating constituents. However, subfractions **C_2, C_4, C_8** and **C_9** gave a time dependent glucose lowering effect up to the fourth hour that was comparable to glibenclamide (5 mg/kg) at all the time points. This indicated that these subfractions had similar minor extrapancreatic and major insulinotropic mechanisms of action of glibenclamide and the compounds for these effects were majorly concentrated in these subfractions [29,33,37] (Table 2). Subfraction **CP_4** comparable to glibenclamide (5 mg/kg) at all the time points, relatively good weight and few TLC spots was further purified to obtain its active compound.

Compound **1** that was identified as β-sitosterol and compound **2,** β-stigmasterol in this study were isolated from the most active column fractions of *Cleistopholis patens* (**CP_4**) and *Sansevieria liberica*, (**C_4**), respectively indicated that they constituted part of the compounds that were responsible for the antihyperglycaemic effect of the plants (Tables 1 and 2). In a similar work, β-sitosterol, isolated from Solanum *surattence* and *Aristolochia indica* has been reported to have a promising antidiabetic effect in drug-induced hyperglycaemia and has been recommended for clinical studies for drug development [38,39]. Similarly, β- stigmasterol isolated from *Bacopa monnieri, Senecio biafrae* and *Gelidium spinosus* has been established to elicit antidiabetic activities both in *in vitro* and *in vivo* studies [40-42]. Also, β- stigmasterol has been reported to be working through both extrapancreatic and insulin stimulating mechanism of actions [40,41] which was similarly confirmed by the result of this study (Table 2). Though β-sitosterol and β-stigmasterol have been isolated from both *Cleistopholis patens* and *Sansevieria liberica* in the earlier studies on this plant [14,18], they have not been identified as the antihyperglycaemic constituents of the plants. This work has therefore shown that these compounds constitute some of the antihyperglycaemic constituents of the plants.

Conclusion

The results of this study concluded that β-sitosterol and β-stigmasterol isolated from the methanolic leaf extract of *Cleistopholis patens* and rhizome of *Sansevieria liberica*, respectively are some of the antihyperglycaemic constituents of the plants may be working through extrapancreatic and insulin stimulating mechanism of actions which justified their use in the management of diabetes mellitus.

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Conflict of Interest

There was no conflict of interests among the co-authors or any other persons in the course of this work.

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